p-ISSN 2615-787X e-ISSN 2615-790X
Accredited by Directorate General of Strengthening for Research and Development No: 30/E/KPT/2018

Tropical Animal Science Journal, March 2021, 44(1):100-107
DOI: https://doi.org/10.5398/tasj.2021.44.1.100
Available online at http://journal.ipb.ac.id/index.php/tasj

Performance of Male Layer Fed Ration Containing Green Algae (*Spirogyra jaoensis*) Extract

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(Received 04-07-2020; Revised 23-09-2020; Accepted 06-10-2020)

ABSTRACT

Some plant extracts containing natural antimicrobial compounds can be used as either feed supplements or alternatives to antibiotic growth promoters (AGP). A plant known as green algae (*Spirogyra jaoensis)*, is one of the species of green algae group. This study aimed to determine the effect of feeding a diet containing *S. jaoensis* ethanolic extract on the performance of male layer. A complete randomized model was used for experimental design in various variables. This study was conducted with 240 male layer day-old chick (DOC) (31.0±1.25 g) divided into 5 treatment groups. Each group consisted of 48 birds, with 3 replicates and 16 birds for each replicate. Dietary treatments tested **namely, Con: control (without *S. jaoensis*), EGA1: Con + *S. jaoensis* extract at 0.05%, EGA2: Con + *S. jaoensis* extract at 0.1%, EGA3: Con + *S. jaoensis* extract at 0.25%, and EGA4: Con + *S. jaoensis* extract at 0.5%. Three chickens from each triplicate were taken and decapitated on the neck and the surgery was performed for histological preparations. The variables observed were villi height, cryptic depth, villi/crypt ratio, goblet cell area of the small intestine, thymus organ weight, *myofiber* area, *fasciculus* area, muscle surface area of *Pectoralis thoracicus* muscle, and growth performance in 14-day-old chickens. Data were subjected to one-way analysis of variance (ANOVA). If it was significant, a Tukey-test was performed with a significance level at p≤0.05. The results showed that small intestinal morphology, muscle morphology, and growth performance of the EGA4 chicken group increased significantly compared to controls (p≤0.05). This study concludes that the administration of *S. jaoensis* ethanolic extract improves morphology of the small intestine, morphometry of thymus organs, *Pectoralis thoracicus* muscle performance, and growth performance in male layer.**

Keywords: Spirogyra jaoensis extract; small intestinal morphology; muscle morphology; growth performance; male layer

INTRODUCTION

Chicken meat is a source of protein containing various amino acids favored more by consumers than fish. According to the Director General of Animal Husbandry and Health (PKH), chicken production increased from 2012-2018 by 8.13% due to the increasing market demand of 6% each year and was expected to increase even more in 2019. Based on these data, an improved method is needed to produce high-quality and high-yielding chicken breeds with highly available nutrition (Ferlito & Respatiadi, 2018).

Layer chicken has a high production value, but for the development of layer chicken farming, various obstacles are often encountered, one of which is the growing issue that tends to be slow. Chicken growth can be influenced by feed efficiency. Feed efficiency is related to the digestion process of feed, which can be studied through the study of the histological structure of a digestive organ that plays a role in the process of absorption of nutrients (Svihus, 2014).

The chicken immune system is another factor that can affect chicken productivity. Male-Layer chickens have lower disease resistance than other types of chickens. Low endurance can lead to a lower production efficiency of layer chicken. The thymus is the primary lymphoid organ that functions to ensure that the environment of the stem cells migrating from the postnatal bone marrow proliferates and differentiates into T lymphocytes (Majumdar, 2018).

The rapid development of science has made many discoveries on methods in studying the initial growth of chickens towards providing the feed. One example is by observing the development of *pectoralis thoracicus* (PT) muscles due to the effect of providing different feeds. PT is the largest portion of the chicken carcass, so the muscle is one of the most important parts of chicken production.

One of the methods carried out by producers to improve the quality and quantity of chickens was to use Antibiotic Growth Promoter (AGP). However, there are side effects of AGP usage. There is the possibility of bacterial resistance in the intestine, residues in tissues, as well as genotoxicity, which can affect animal health and production results. If consumed by humans, it can be harmful to the body.

One of the efforts to improve the productivity and immunity of the layer male chickens is to mix the basic feed by increasing additional feed from herbal plants. For an alternative, green algae have the potential as one of the natural immunomodulators for livestock. One herbal ingredient that contains natural antimicrobial compounds is *Spirogyra jaoensis*. *S. jaoensis* is a green alga that can grow in Indonesia. *Spirogyra sp.* is a green alga or Chlorophyta containing gallotannin, saponin, phenolic, and flavonoid compounds, which can be used as an antimicrobial because its components can inhibit microbial growth (Champa *et al*., 2015). According to the research by Saragih *et al*. (2019), supplementing feed powder with *S. jaoensis* can improve small intestine morphology, pectoralis muscle growth, and growth performance in broiler chickens.

However, there is limited information regarding the effect of *S. jaoensis* ethanolic extract as a growth supplement and enhancement of the body's defense system of male layer. Therefore, the study was conducted to evaluate its effects on the digestive organs of the small intestine and the weight of lymphoid organs in male layer and determine the effectiveness of *S. jaoensis* ethanolic extract as the natural AGP alternative on PT muscle performance and growth performance.

MATERIALS AND METHODS

Preparation of *Spirogyra jaoensis* Ethanolic Extract

The preparation of *S. jaoensis* ethanolic extract was conducted in the Integrated Laboratory of Research and Testing (LPPT) at Universitas Gadjah Mada (UGM), Yogyakarta-Indonesia with an extraction method. *S. jaoensis* was washed with distilled water, then drained and sliced into small pieces. *S. jaoensis* was dried in the sunlight for 1 day and then dried again with an incubator at 56°C for 3 days. The dried *S. jaoensis* was then mashed with a blender to facilitate the extraction process. The powder was then macerated with 96% ethanol. The liquid extract was then evaporated with a vacuum evaporator at 60°C for 3 days until freed from ethanol solvent and became viscous (Kementerian Kesehatan Republik Indonesia, 2017).

Experimental Animals and Diets

The protocol of the male layer study was approved by the commission for Clinical Experiment the Integrated Laboratory of Research and Testing (LPPT) at Universitas Gadjah Mada (UGM), Yogyakarta-Indonesia. The commission issued the ethical clearance certificate with the number of 00039/04/LPPT/IV/2018. The materials used in this study were 240 male layer day-old chick (DOC), basal feed (Table 1), ethanolic extract of *Spirogyra jaoensis*, Medivac ND Hitcner B1, materials for histological slide preparations using the paraffin method, Periodic Acid Schiff, and Alcian Blue (PAS-AB) dye, haematoxylin Ehrlich dye, and eosin Y 1%. Experimental birds were kept in 5 cages. Other supporting tools were 1 set for histological slide preparation with paraffin method, staining jar, light microscope, ImageJ, and Image Raster. Chicken feed used was basal feed which had been added with the *S. jaoensis* ethanolic extract with various concentrations. Feed production began with dissolving *S. jaoensis* ethanolic extract in warm water.

Experimental Design and Treatments

A completely randomized model was used for the experimental design of various variables. The basal feed was mixed with a solution of *S. jaoensis* ethanolic extract with the following concentrations: Con (Control) was

Table 1. Basal feed formulation and nutrition content

Composition of feed (%)	Single feed
Corn	49.0
Soybean meal	29.0
Rice bran	9.8
Full-fat soya	5.4
Crude palm oil	3.0
Dicalcium phosphate	2.37
Premix vitamin[a]	0.03
Premix mineral[b]	0.06
D, L-methionine	0.22
NaCl	0.32
Calcit	0.5
L-lysine HCl	0.1
L-threonine	0.04
Choline chloride 60%	0.16
Calculated composition[c]	
Metabolizable energy of poultry (kcal/kg)	2,904.02
Crude protein (%)	20.23
Crude fat (%)	8.30
Fiber (%)	3.37
Lysine (%)	1.22
Methionine (%)	0.53
Methionine + cysteine (%)	0.86
Calcium (%)	1
Phosphorus, total (%)	0.95
Phosphorus, available (%)	0.5
Sodium (%)	0.15
Chloride (%)	0.23

Note: [a]Premix vitamin provided the following per kilogram of diet (vitamin A: 15000 IU, vitamin D3: 3000 IU, vitamin E: 22.5 mg, vitamin K3: 3 mg, vitamin B1: 3 mg, vitamin B2: 9 mg, vitamin B6: 4.5 mg, vitamin B12: 30 mcg, biotin: 30 mcg, folic acid: 1.5 mg, niacin: 45 mg, pantothenic acid: 1.5 mg, vitamin C: 0 mg, choline: 2090 mg & 1242 mg); [b]Premix mineral provided the following per kilogram of diet (Cu: 12 mg, Fe: 72 mg, Iodine: 0.9 mg, Mn: 84 mg, Se: 0.3 mg, Zn: 60 mg); [c]Proximate, amino acids, minerals, and metabolizable energy were obtained from calculated values (Hartadi *et al*., 2017).

basal feed without *S. jaoensis* ethanolic extract; EGA1 (green algae extract 1) was basal feed with *S. jaoensis* ethanolic extract in 0.05%; EGA2 (green algae extract 2) was basal feed with *S. jaoensis* ethanolic extract in 0.10%; EGA3 (green algae extract 3) was basal feed with *S. jaoensis* ethanolic extract in 0.25%; EGA4 (green algae extract 4) was basal feed with *S. jaoensis* ethanolic extract in 0.50%. A total of 240 male layer DOCs were kept intensively in a 200L cage equipped with a bulb to keep it warm for 14 days and were divided into 5 treatment groups, and each consisted of 48 chickens. Each group consisted of 3 replicates with 16 chickens. Cage lighting was provided 24 hours a day until the chicken was 10 days old.

Chicken Rearing Procedure

The day after hatched, the chickens were acclimated in advance for 2 days. Chickens were given the first treatment at the age of 3 days. The chickens were vaccinated through the right eye with Medivac ND Hitcner B1 on the third day. All the chicks were weighed at the ages of post-hatch, 4, 6, 9, 12, and 14 days old. The next day before being fed, the remainder of the previous day's feed was weighed and recorded to calculate the amount of feed consumed (feed intake). The feed conversion ratio (FCR) was calculated as the feed intake needed to gain 1 kg in body weight.

Variables and Measurement Method

Fourteen days old chicken fasted for 6 hours, 3 chickens from each triplicate were taken and decapitated on the neck, and the surgery was performed for the removal of the small intestine, thymus, and PT muscle organs. In the histological structure analysis of the small intestine, the data obtained were in the form of villi height, crypt depth, and goblet cells area, while in the histological structure analysis of the PT muscle, the data obtained were in the form of muscle weight, muscle area, myofiber, and PT fasciculus. Each organ was measured by analytical weight. The results were then recorded systematically. Furthermore, histological preparations were made using Periodic Acid Schiff Alcian Blue (PAS-AB) staining for small intestinal organs and the paraffin method with HE (Hematoxylin-Eosin) staining for PT muscle organs.

The histological slide was observed using a light microscope. Documentation of slide preparations was taken using the Leica microscope digital camera and application. In a small intestinal histological slide, villi height, crypt depth, and goblet cell area were observed. In observing villi height and crypt depth, a magnification of 10x10 was used. Villi and crypt were sought with five different fields of view in each preparation, then documented. The villi height and crypt depth of the documented slide preparations were then measured with a unit length of μm using the ImageJ application. The ratio between the villi height and crypt depth was calculated by the following formula (Incharoen *et al*., 2010).

Villi/crypt ratio= average length of villi (μm) / average depth of crypt (μm)

The measurement area of the goblet cells used a magnification of 40x10. Goblet cells were measured in different villi in 5 fields of view in each slide preparation. Measurements were made through goblet cells measured at the edges surrounding the cell using the Image Raster application with a unit area of μm^2. Meanwhile, in the PT muscle histology slide preparations, 1 slide contained coupes, each of which had 5 fasciculi. Each fasciculus was observed for 5 myofibers. Measurements of muscle area, myofiber area, and fasciculus were conducted using ImageJ software.

Statistical Analysis

The data were analyzed with SPSS 23.0 (SPSS, Inc., Chicago, IL, USA) for Windows using a one-way ANOVA procedure and presented as mean ± SEM. Significant differences among means were evaluated by Tukey's comparison test at $p \leq 0.05$.

RESULTS

Male layers given a basal diet with the addition of *S. jaoensis* ethanolic extract were weighed at 1, 4, 6, 9, 12, and 14 days and then divided into five groups. Table 2 shows the results of the one-way ANOVA analysis from duodenal data in the form of villi height, crypt depth, the ratio of villi height and crypt depth, and the area of goblet cells. In a 14-day-old male layer, the results showed that there were significant differences in the height of the duodenal villi (Figure 1), whereas the crypt depth and the ratio of villi height to crypt depth did not show significant results. However, the crypt depth continued to show an increase in numbers as the addition of the *S. jaoensis* ethanolic extract concentration in the basal feed even though it was not significant. Based on these data, the variables used in this study, i.e., villi height, crypt depth, and goblet cell area, showed increases in values at each concentration of the feed treatment.

Table 2 shows the results of one-way ANOVA analysis from jejunum data in the form of villi height, crypt depth, the ratio of villi height and crypt depth, and the area of goblet cells. In a 14-day-old male layer, the results showed that there were significant differences in almost all variables used, namely villi height, crypt depth, and goblet cell area. Each variable showed an increase in the value directly proportional to the addition of the concentration of *S. jaoensis* ethanolic extract in the basal feed.

Table 3 shows that thymus weight and thymus index in the control group showed more significant results in EGA1 group compared to EGA2, EGA3, and EGA4 groups, namely the addition of *S. jaoensis* ethanolic extract by 0.05%. This result showed that there was an effect of *S. jaoensis* ethanolic extract on the thymus weight and thymus index.

Table 4 shows that there were significant differences in the fascicular area between the controls with

Table 2. Villus height, crypt depth, V/C ratio, and goblet cells area of small intestine on male layer with the addition of ethanolic extract of *Spirogyra jaoensis* at 14 days old

Variables	Treatments				
	Con	EGA1	EGA2	EGA3	EGA4
Duodenum					
Villus height (µm)	799.45±22.37^{a}	823.69±62.41^{a}	796.63±74.07^{a}	872.07±36.23^{ab}	949.00±89.04^{b}
Crypt depth (µm)	140.98±5.30^{ns}	144.11±12.13^{ns}	146.88±5.08^{ns}	153.16±9.60^{ns}	155.71±6.19^{ns}
V/C ratio	5.69±0.51^{ns}	5.76±0.76^{ns}	5.43±0.54^{ns}	5.71±0.34^{ns}	6.11±0.72^{ns}
Area of goblet cells (µm²)	18.67±2.69^{a}	22.15±1.54^{ab}	26.70±4.21^{bc}	26.90±1.87^{bc}	32.62±7.10^{c}
Jejunum					
Villus height (µm)	446.52±126.46^{a}	522.69±64.32^{ab}	620.60±46.62^{ab}	656.84±168.80^{ab}	681.67±152.35^{b}
Crypt depth (µm)	91.72±6.99^{a}	125.23±2.47^{b}	132.33±11.68^{b}	132.74±28.36^{b}	143.64±13.01^{b}
V/C ratio	4.81±1.05^{ns}	4.17±0.47^{ns}	4.70±0.19^{ns}	4.92±0.52^{ns}	4.70±0.63^{ns}
Area of goblet cells (µm²)	31.75±6.30^{a}	38.46±5.95^{ab}	50.41±5.16^{bc}	54.08±10.33^{c}	53.13±7.25^{c}
Ileum					
Villus height (µm)	415.17±9.07^{a}	435.40±12.61^{ab}	463.08±7.84^{bc}	486.63±16.72^{c}	521.81±22.48^{d}
Crypt depth (µm)	99.25±3.01^{a}	101.40±1.99^{a}	114.14±2.69^{ab}	114.92±13.28^{ab}	126.90±9.36^{b}
V/C ratio	4.19±0.16^{ns}	4.29±0.13^{ns}	4.06±0.09^{ns}	4.27±0.45^{ns}	4.13±0.43^{ns}
Area of goblet cells (µm²)	32.44±1.07^{a}	41.74±5.96^{a}	40.09±4.92^{a}	59.49±7.20^{b}	55.97±4.37^{b}

Note: V/C=Villus height/crypt depth; Means in the same row with different superscripts differ significantly (p≤0.05); ns= not significant; Con= Control group with basal feed; EGA1= Chicks treated with basal feed + *Spirogyra jaoensis* (0.05%); EGA2= Chicks treated with basal feed + *S. jaoensis* (0.1%); EGA3= Chicks treated with basal feed + *S. jaoensis* (0.25%); EGA4= Chicks treated with basal feed + *S. jaoensis* (0.5%).

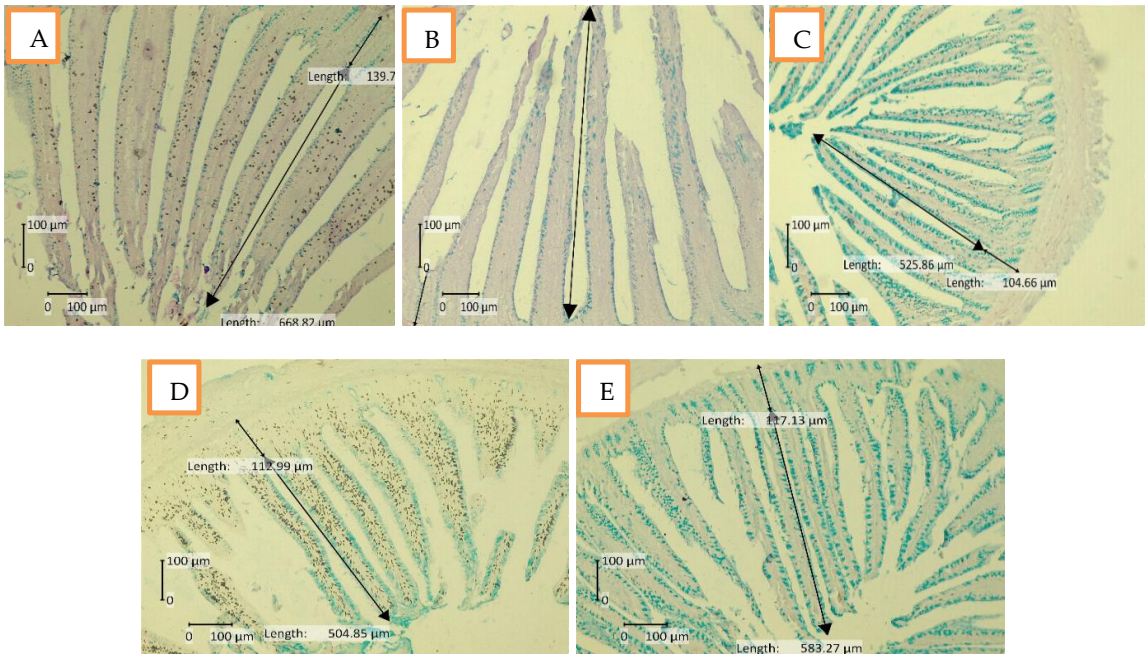

Figure 1. Histological structure of small intestine of male layer in each treatment group of ethanolic extract of *Spirogyra jaoensis* on basal feed at 14 days old. Con= Control group with basal feed; EGA1= Chicks treated with basal feed + *Spirogyra jaoensis* (0.05% of feed); EGA2= Chicks treated with basal feed + *S. jaoensis* (0.1%); EGA3= Chicks treated with basal feed + *S. jaoensis* (0.25%); EGA4= Chicks treated with basal feed + *S. jaoensis* (0.5%). In the control, the villi are shorter and thinner than those in chicks fed with ethanolic extract of *S. jaoensis*.

EGA2, EGA3, and EGA4 groups. The fascicular area of the EGA2 group also showed significant differences from EGA3 and EGA4 groups. However, there was no significant difference between the control and EGA1 group. In the myofiber area calculation, the control was significantly different from the other treatments. EGA1 group was significantly different from EGA2, EGA3, and EGA4 groups. EGA2 group looked significantly different from EGA3 and EGA4 groups, while EGA3 group was significantly different from EGA4 group (Figure 2).

The variables of growth performance in male layer were weight gain, feed consumption, and feed conversion ratio (FCR): The growth patterns of the male layers are shown in Figure 3. There was an increase in

Table 3. Growth of thymus weight (g) and thymus index in male layer with the addition of ethanolic extract of *Spirogyra jaoensis* at 14 days old

Variables	Treatments				
	Con	EGA1	EGA2	EGA3	EGA4
Timus weight (g)	0.22±0.048[a]	0.35±0.023[b]	0.34±0.062[ab]	0.30±0.072[ab]	0.31±0.069[ab]
Index of thymus	0.25±0.053[a]	0.37±0.021[b]	0.36±0.062[ab]	0.32±0.079[ab]	0.33±0.064[ab]

Note: Means in the same row with different superscripts differ significantly (p≤0.05); ns= not significant; Con= Control group with basal feed; EGA1= Chicks treated with basal feed + *Spirogyra jaoensis* (0.05%); EGA2= Chicks treated with basal feed + *S. jaoensis* (0.1%); EGA3= Chicks treated with basal feed + *S. jaoensis* (0.25%); EGA4= Chicks treated with basal feed + *S. jaoensis* (0.5%).

Table 4. Muscle performance of *Pectoralis thoracius* of male layer in each treatment group of the addition of ethanolic extract of *Spirogyra jaoensis* at the age of 4, 6, 9, 12, and 14 days

Variables	Treatments				
	Con	EGA1	EGA2	EGA3	EGA4
Muscle weight (g)	3.15±0.45[a]	3.51±0.37[a]	3.52±0.47[a]	3.53±0.29[a]	3.55±0.51[a]
Muscle area (cm²)	9.65±1.33[a]	9.75±1.03[a]	10.20±0.81[a]	10.37±0.97[a]	10.89±0.87[a]
Fasciculus area (µm²)	27033.44±4779.09[a]	28776.48±2639.60[ab]	37043.28±5102[bc]	41225.77±7230.92[c]	42290.85±4737.68[c]
Myofiber area (µm²)	96.52±2.3[a]	113.48±1.62[b]	137.11±5.60[c]	183.19±12.14[d]	209.73±1.8[e]

Note: Means in the same row with different superscripts differ significantly (p≤0.05); ns= not significant; Con= Control group with basal feed; EGA1= Chicks treated with basal feed + *Spirogyra jaoensis* (0.05%); EGA2= Chicks treated with basal feed + *S. jaoensis* (0.1%); EGA3= Chicks treated with basal feed + *S. jaoensis* (0.25%); EGA4= Chicks treated with basal feed + *S. jaoensis* (0.5%).

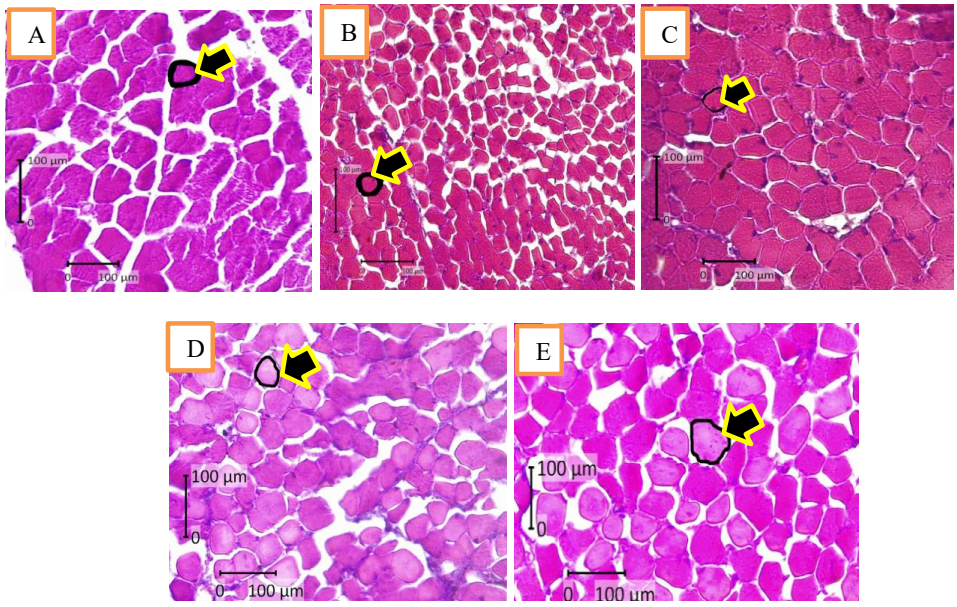

Figure 2. Cross-section of the myofiber area of *Pectoralis thoracicus* muscle of male layer in each treatment group of ethanolic extract of *Spirogyra jaoensis* on basal feed at 14 days old. Con= Control group with basal feed; EGA1= Chicks treated with basal feed + *Spirogyra jaoensis* (0.05% of feed); EGA2= Chicks treated with basal feed + *S. jaoensis* (0.1%); EGA3= Chicks treated with basal feed + *S. jaoensis* (0.25%); EGA4= Chicks treated with basal feed + *S. jaoensis* (0.5%). In the control, the myofiber area is smaller than those in chicks fed with *S. jaoensis* ethanolic extract.

body weight of the male layer in the control group as compared to the EGA4 treatment group for 14 days of observation.

In Table 5 it was clear that EGA4 group had the highest body weight and the lowest feed intake for each treatment. The results showed that the EGA4 group treated with the addition of *S. jaoensis* ethanolic extract with the highest concentration into the optimal basal feed improved the growth performance of the male layer. The FCR value of EGA4 group also had the lowest value of each treatment.

DISCUSSION

Feed efficiency is related to the digestion process of feed, which can be studied through the study of the histological structure of a digestive organ that plays a role in the process of absorption of nutrients, one of which is

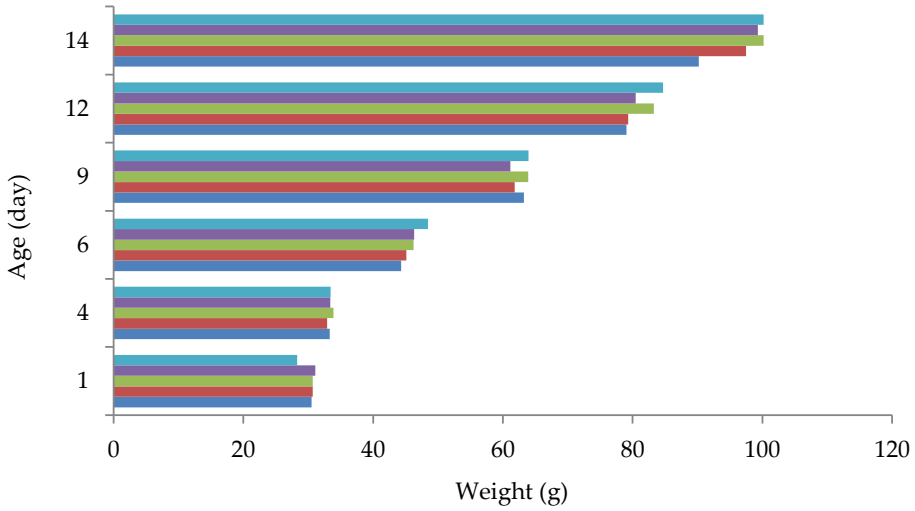

Figure 3. Weight of male layer in each treatment group of ethanolic extract of Spirogyra jaoensis on basal feet at day 1, 4, 6, 9, 12, and 14 days. Con= Control group with basal feed; EGA1= Chicks treated with basal feed + *Spirogyra jaoensis* (0.05% of feed); EGA2= Chicks treated with basal feed + *S. jaoensis* (0.1%); EGA3= Chicks treated with basal feed + *S. jaoensis* (0.25%); EGA4= Chicks treated with basal feed + *S. jaoensis* (0.5%). EGA4 EGA3 EGA2 EGA1 Con.

Table 5. Growth performance of male layer in each treatment group of the addition of ethanolic extract of *Spirogyra jaoensis* at the age of days 4, 6, 9, 12, and 14

Variables	Age (days)	Treatments				
		Con	EGA1	EGA2	EGA3	EGA4
Body weight (g)	Posthatch	30.5±1.08^{a}	30.7±1.33^{a}	30.7±1.05^{a}	31.1±1.52^{a}	31.86±1.29^{a}
	4	33.3±2.26^{a}	32.9±2.64^{a}	33.9±1.72^{a}	33.4±2.71^{a}	33.44±1.87^{a}
	6	44.3±2.44^{a}	45.1±3.68^{a}	46.22±3.73^{a}	46.33±2.91^{a}	48.44±2.83^{a}
	9	63±4.26^{a}	62.5±5.06^{a}	65.5±5.52^{a}	61.5±6.2^{a}	65.8±6.7^{a}
	12	78.9±4.62^{a}	79.5±8.23^{a}	83.9±6.19^{a}	80.7±4.69^{a}	85.7±6.9^{a}
	14	90.2±3.73^{a}	97.5±4.62^{ab}	100.2±7.7^{b}	99.3±5.51^{b}	100.2±6.33^{b}
Feed intake (g/day)		13.1±2.72^{a}	12.51±2.37^{a}	11.84±2.49^{a}	11.19±2.75^{a}	10.87±3.56^{a}
Weight gain (g/day)		5.69±2.70^{a}	6.02±2.69^{a}	6.63±2.40^{a}	6.59±3.27^{a}	6.68 ±2.67^{a}
FCR		3.25±3.28^{a}	3.65±5.44^{a}	2.2±1.61^{a}	2.31±1.79^{a}	1.98 ±1.35^{a}

Note: Means in the same row with different superscripts differ significantly ($p \leq 0.05$); ns= not significant; Con= Control group with basal feed; EGA1= Chicks treated with basal feed + *Spirogyra jaoensis* (0.05%); EGA2= Chicks treated with basal feed + *S. jaoensis* (0.1%); EGA3= Chicks treated with basal feed + *S. jaoensis* (0.25%); EGA4= Chicks treated with basal feed + *S. jaoensis* (0.5%); FCR= feed conversion ratio.

the small intestine (Svihus, 2014). All the main nutrients are mostly digested and absorbed in the jejunum. The jejunum has a smaller size than the duodenum but larger than the ileum. The absorption time in the jejunum is around 40 to 60 minutes, longer than the absorption in the ileum. This difference is caused by the amount of material absorbed by the jejunum is greater than the ileum. The absorption of nutrients such as fat, starch, and protein is mostly done in the jejunum (Svihus, 2014). Villi in the jejunum are covered by a layer of columnar epithelium, which is shorter and wider than the duodenum. Most villi in the jejunum have a blunt end and a broad base (Nasrin *et al.*, 2012).

Flavonoid is one of the antimicrobial compounds that have a role in increasing the villi height and crypt depth by reducing the number of pathogenic bacteria in the small intestinal wall and reducing the toxic compounds produced by microbes that can damage the intestinal epithelial cells and cause the villi and crypt to be shorter (Xie *et al.*, 2015). One herbal ingredient that contains natural antimicrobial compounds is *Spirogyra jaoensis*. *S. jaoensis* has antimicrobial activity because it contains flavonoid, tannin, saponin, and phenolic components that can inhibit microbial growth (Champa *et al.*, 2015). *S. jaoensis* extract, which has a natural compound in the form of flavonoids, can protect the stomach wall and small intestine of male layer chicken. With the protection of the chicken stomach and small intestine, the process of nutrients absorption from feeds will increase. Based on Table 2, the goblet cell area showed an increase in results between the control group and EGA4 group. Mucin, which is secreted by the goblet cells, is very important to help the absorption of food substances. The amount of mucin fluid in the goblet cells can be estimated from the area of the goblet cells. The mucin fluid secreted by the goblet cells will fa-

cilitate the absorption of nutrients in the small intestine (Birchenough *et al.,* 2015).

Growth performance is closely related to muscle development, and the amount of good nutrition also affects both growth performance and muscle development. If the nutrients provided are not optimal, it will interfere with the growth of the chicken body and the development of chicken muscles (Saragih & Daryono, 2012). According to the previous research by Saragih *et al*. (2019), feed powder supplemented with the *Spirogyra jaoensis* can improve small intestine morphology, pectoralis muscle growth, and growth performance in broiler chickens. The nutrient contents of *S. jaoensis,* such as protein, carbohydrate, and lipid, can also influence the growth performance of broiler chicken.

Skeletal muscle formation in chickens will be perfect when the chicken has hatched. Muscle growth is influenced by myofiber. The myofiber will increase in length and size so that muscle weight is directly proportional to the number of myofibers. The higher the numbers of myofiber will increase the hypertrophy of muscle cells. Enlargement of muscle fibers, namely hypertrophy, increases the production of myofibrils, mitochondria, sarcoplasmic reticulum, and other organelles because the hypertrophic muscles contain a higher number of myofibrils. The hypertrophy activities of muscle cells depend on the stimulation of IGF-1 hormone (Insulin-like Growth Factor-1). The IGF-1 hormone works directly on the target cell so that the growth and development of soft tissue and bone tissue also accelerate the absorption of protein. The IGF-1 hormone is known as one of the more dominant hormones needed to support the normal growth in chickens (Anh *et al*., 2015). IGF-1 hormone has an anabolic role in the process of growth and repair of skeletal muscle, and this hormone will also increase the lean muscle mass while reducing adipose tissue (Davis *et al*., 2015). Flavonoids can also increase protein synthesis in muscles and stimulate the growth with the mechanism that IGF will bind with receptor-enzymes so that the effector protein in the target cell will be active and tyrosine is phosphorylated in protein (Bikle *et al*.,2015). With the results in Figure 1 and Figure 2, it can be seen that the flavonoid nutrition contained in the ethanolic extract of *S. jaoensis* increases the development of the small intestine and muscles.

Fibers in *S. jaoensis* also had a positive impact on the intestine morphology that eventually improved nutrient absorption in the small intestine. It shows that the energy metabolism of *S. jaoensis* can increase chicken growth and the efficiency of the digestive system (Saragih *et al*., 2019). These results were consistent in our study that the addition of *S. jaoensis* could enhance and improve the histological structure of the small intestine, the morphometry of the thymus organ, PT muscle performance, and growth performance of male layer.

Apart from flavonoids, the antioxidant compounds in the ethanolic extract of *S. jaoensis* also have a close relationship with the immune system. This compound can act as a protective immune cell against damage caused by free radicals (Puertollano *et al.,* 2011). From this research, the addition of ethanolic extract of *Spirogyra jaoensis* at a concentration of 0.05% can increase the thymus weight and the index of thymus organ (Table 3). The weight of a lymphoid organ can indicate the mass of its constituent tissue so that it can be related to the body's ability to provide immunocompetent cells in lymphoid tissue during the immune response while the organ index is the ratio between organ weight to body weight (Makram *et al.,* 2010). With the existence of immunity against pathogens, flavonoids can protect the digestive system so that the process of absorption of food runs smoothly. This condition improves the immune systems and growth performances of experimental chicken (Panche *et al.,* 2016). Compound with immune activity to pathogens contained in flavonoids can also improve the growth performance of chicken and increase muscle weight and muscle expansion of the male layer chicken (Yazdi *et al*., 2014).

The variables of growth performance in male layer were weight gain, feed consumption, and feed conversion ratio (FCR). The success of growth performance was also marked by the increasing average body weight, increasing body weight every day, low daily feed intake, and low FCR values. The lower FCR value indicated a better food conversion also the success of the cultivation technique and financially. Low FCR means low feed costs but makes a great proportion of chicken weight (Yi *et al*., 2018).

The study was determining the effectiveness of the addition of ethanolic extract of *S. jaoensis* as the natural AGP alternative on PT muscle performance and growth performance because of its effect on increasing the digestive organs of the small intestine and the weight of lymphoid organs, especially the thymus in male layer. Ethanolic extract of *S. jaoensis* can also be used as a supplement in a basal feed of the male layer based on the histological structure of muscles and small intestine, which shows positive results in increasing the process of optimal nutrient absorption.

CONCLUSION

The addition of ethanolic extract of *Spirogyra jaoensis* increases the length of villi, ratio of villi/crypts, and area of goblet cells in the of the small intestine, weight and index of thymus organs, the fascicular and myofiber area of *Pectoralis thoracicus* muscle, and growth performance in male layer.

CONFLICT OF INTEREST

The authors declare that they have no potential conflict of interest with other people related to the material discussed in the manuscript.

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